Effects of Cell-to-cell interaction on de-differentiation of chondrocytes

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Experiments run with lab partner, Layla Barkal. All methods following protocols from http://openwetware.org/wiki/20.109(S09):Module_3

Introduction:

Scientists are taking advantage of tissue engineering applications to find suitable treatments of diseases related to cartilage. Some methods of tissue regeneration include inserting scaffolds that carry stem or mature cells, including other molecules. The mature cells present in cartilage tissue are called chondrocytes, which can be grown in vitro before being inserted into the body. However, the environment the cells are in, especially in vitro, could cause the cells to de-differentiate back to fibroblasts. So, it is necessary to learn more about the effects of the environment on the phenotype of the chondrocytes before we can use these cells in tissue engineering applications.

We wanted to test, specifically, the effects of cell interaction on de-differentiation of chondrocytes in vitro. The experiment was designed to learn about the differences between the levels of de-differentiation in a sample where the cells were clustered closer together, and another where they were scattered, with less interaction.

Sigma Aldrich 1% alginate, a hydrogel, was used for the experiment where the control was done by mixing chondrocytes with alginate before the alginate chains were polymerized into beads, so, the cells would end up trapped between the chains, far apart. In the experiment sample, however, the alginates beads were made first without any cells, and then cells were injected later into the beads using syringes. Using this method, we would expect cells to stay clustered in the little puncture that had been made in the bead. We expect that with the beads closer to each other, cell-to-cell interaction will increase, making the environment more similar to that the cells grow in, in vivo. As a result, the de-differentiation might decrease in the chondrocytes compared to that in the cell of the control. In addition to the beads, our third sample was a monolayer of chondrocytes, which are expected to de-differentiate as well, according to Brodkin 2004.

Collagen type I and type II transcripts and protein levels were assayed to tell us more about the differentiation of the cells, as chondrocytes express more collagen II while stem cells express collagen I.

Results:

Cell viability assay. Once the experiment was set up, we wanted to study cell viability using the Live/Dead fluorescence assay. However, because while we were injecting our beads with cells, not all of the beads were injected, we were unable to depend on this assay were unable to get any verifiable results on cell viability. We had also started with a small total number of cells (10^6 cells) for all three samples. Nonetheless, we were able to get results for both collagen I and II transcripts and proteins for all samples, proving there were indeed cells in the control and experimental beads.

RT-PCR indicated that the experimental cells de-differentiated less than the control. An RT-PCR reaction was run to reverse transcribe collagen I and II mRNA and amplify the transcripts. Collegen I and II primers were designed and 80ng of mRNA, extracted from the cells from each sample, were used in the RT-PCR reactions. Although the A260/A280 ratios for the experimental and monolayer of cells samples, did indicate a protein contamination, we still got collagen I and II transcripts, which appeared when run on a gel. ImageJ analysis was used to measure the mean intensity of the same area (from the center) of the bands in the RT-PCR electrofluoresis gel (Figure 1). By looking at the same area for all the bands and after the background mean intensity was subtracted from the data and the collagen II band intensity was normalized to that of GAPDH, we were able to compare the intensities of the bands and calculate the collagen II to I ratios. The ratios were 0.04, 1.42 and 0.33 for the control, the experimental sample and in the sample of monolayer cells, respectfully. From these numbers, it seems that many of the cells in the control were de-differentiated since collagen I was present in greater amounts than collagen II, just as expected. In the experimental example, the ratio was much higher; meaning a lot less cells had de-differentiated, as we had hoped. The ratio for the cells from the monolayer of cells was higher than that for the cells in the control beads but still lower than the ratio in the experimental cells. Which means when the cells were closer to each other, they had de-differentiated less than when they were separated, but more than when they were clustered together in a more 3D structure.

ELISA protein assay gave different results. Collagen I, II proteins were extracted from the cells and the ELISA assay was used to calculate their concentrations. Standards with known concentrations were used to make a calibration curve, to be used in calculating the concentrations of collagen I and II in our samples, from the absorbance data (Figure 2). For both collagen I and II, we got measurable amounts of protein, since they were higher than the concentration of the lowest standard used, although they were not very high concentrations. The collagen II to I ratios we got for the control, experimental and monolayer cells samples were, 0.42, 0.33, 0.22, respectfully. These results are not compatible with the ratios we got from the transcripts.

Discussion:

We wanted to study the effects of cell-to-cell interaction on de-differentiation in chondrocytes. We proposed an experiment where the cells would be injected into alginate beads, to keep the cells closer to each other, as opposed to the standard method, where the cell are trapped, and scattered between the polymerized, alginate chains and a third sample with just a monolayer of cells. We assayed the levels of collagen type I and type II transcript and protein levels as cell type markers and calculated the collagen II to collagen I ratios. The transcript results we got from the RT-PCR, gave us the desired results, where the cells scattered in alginate beads were de-differentiated the most, followed by the monolayer of cells, and the least de-differentiated was the experimental sample, with the cells more clustered in the puncture made in the bead. Although we got the results we had hoped for, the experiment must be repeated before we can make definite conclusions. The fact that the ALISA assay gave different results for the ratios, stress the fact that we cannot depend on our transcript data. When the experiment is repeated, we must make sure that only beads that were injected with cells are included in our analysis. It is also important to be able to get results from a viability assay before we can continue our experiment. We should also try to get higher levels of transcripts and proteins, which may have affected our assays. If we do get more dependable results with further research, this information can be used to enhance tissue regeneration treatments in the future.

Reference:

Brodkin et.all; *Chondrocyte phenotypes on different extracellular matrix monolayers*; Elsevier, Biomaterials 25 (2004).

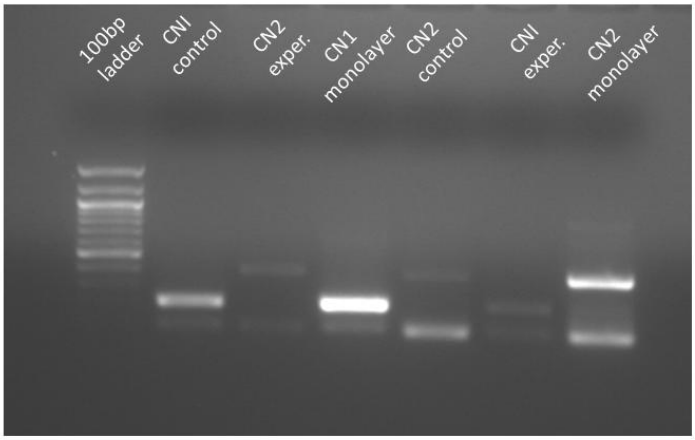

Figure 1: RT-PCR products of collagen I, II transcripts run on an electrofluoresis gel.

Collagen I and II transcripts made through RT-PCR reactions were run on an agarose gel. The top band in each lane represents either collagen I or II, depending on the lane, and the second band is GAPDH. Notice that the CNI and II experimental samples lanes were switched from what would have been a reasonable order.

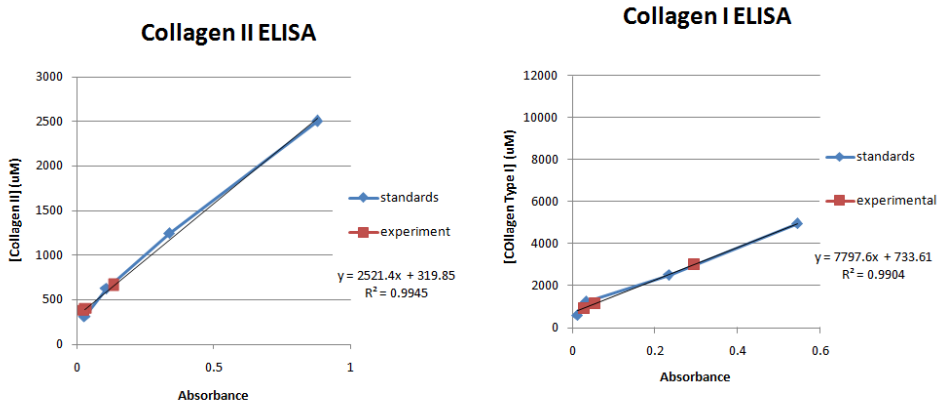

Figure 2: ELISA analysis for collagen I and II protein concentrations.

Standards with known concentrations (blue dimonds) were used to make a calibration curve, to be used in calculating the concentrations of collagen I and II in our samples (red squares), from the absorbance data.